Structure and unique mechanical aspects of nuclear lamin filaments

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Structure and unique mechanical aspects of nuclear lamin filaments

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The major constituent of the nuclear envelope is the nuclear lamina. A fibrous meshwork of lamin filaments spanning underneath the nuclear membrane provides mechanical support to the nucleus. Lamins, type V intermediate filament proteins, are also involved in many other nuclear activities such as DNA repair and transcription. Recent structural studies provide new insight into how lamins assemble into ~3.5 nm thick filaments, which is in contrast with cytoplasmic intermediate filaments. The thinnest component of the cell cytoskeleton exhibits surprising mechanical properties. Here, we critically review and discuss structural and mechanical aspects of single lamin filaments.

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Introduction

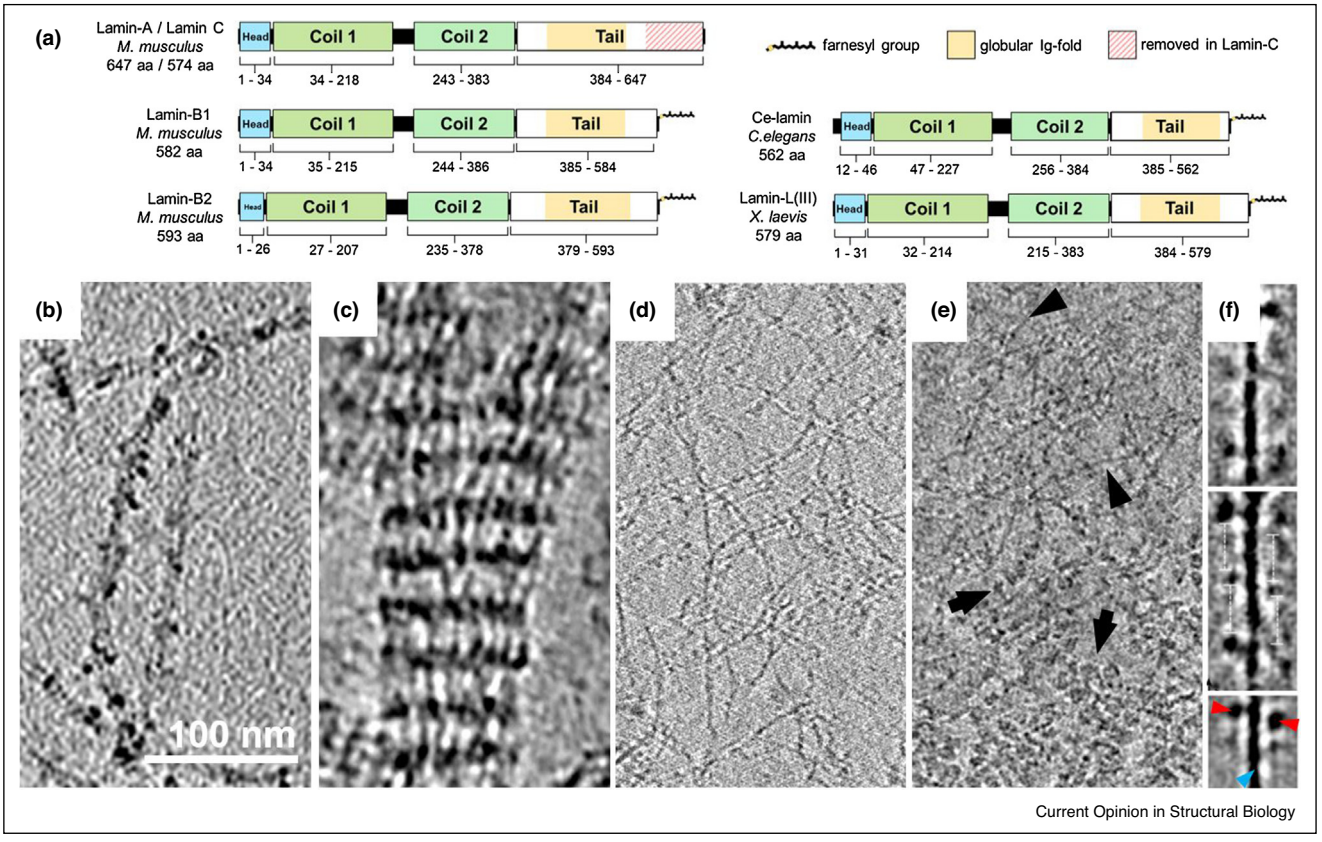
The inner and outer nuclear membranes (INM and ONM, respectively) provide a physical barrier that is perforated by the nuclear pore complex (NPC), an enormous macromolecular complex that provide the sole gateways in and out of the nucleus [1,2]. Attached to the INM and interacting with NPCs, the nuclear lamina [3–6] provides a layer of filamentous scaffolding that is anchored to the membrane as well as to the NPCs. Composed of lamins and lamin-associated proteins, which include emerin and LBR among others [7,8], the 10−30 nm thick nuclear lamina is found ubiquitously in differentiated cells.

Over the years it has been shown that lamins play a role in many nuclear functions, for example, transcription regulation, replication, DNA repair and chromatin organization [7,9–12]. Four main lamin isoforms are found in mammals. In humans, they are encoded by the *LMNA*, *LMNB1*, *LMNB2* genes to express lamin A/C, lamin B1 and lamin B2, respectively. While the B-type lamins are expressed in all types of mammalian cells, the expression of A-type lamins is developmentally regulated and appears only during cell differentiation [13,14]. Notably, the expression of A-type and B-type lamins is highly variable and depends on the cell type and differentiation stage [15]. Surprisingly, recent studies showed that differentiated cells lacking B-type lamins are viable [16]. In differentiated cells, both types of lamins are localized to the nuclear periphery; however, small amounts of A-type lamins are also found in the nucleoplasm and are suggested to have a major functional impact on chromatin organization and gene regulation [17].

Mutations in lamin A/C have been directly associated with >15 different diseases, termed laminopathies [18]. Laminopahties are highly varied and include muscular dystrophies [19], lipodystrophies [20] and progeria syndromes [21] to name a few. However, it is still not clear, how the single point mutations can lead to such a wide variety of diseases and it remains an open and heavily studied question [5]. Many of these mutations affect tissues under high mechanical load like muscles and heart [22,23]. This may suggest that mutations also alter the mechanical properties of lamins in addition to alteration of their structure and interaction with other proteins [24,25].

Lamins are classified as type V intermediate filament (IF) proteins and are considered to be the ancestors of cytoplasmic IFs [26]. Similar to other IF proteins, lamins contain a long rod domain which comprises four coiled-coil α-helical segments, termed 1A, 1B, 2A and 2B, separated by flexible linkers (Figure 1a). This domain is flanked by a non-helical N-terminal head and C-terminal tail domain. The C-terminal domain hosts a nuclear localization signal (NLS), an immunoglobulin (Ig) like fold and a CaaX motif box (C, cysteine; a, aliphatic residue; X, any residue). Lamins are post-translationally modified at the CaaX motif by farnesylation of the cysteine residue (except for lamin C, which is devoid of the CaaX motif box). Lamin A is further cleaved before the -aaX sequence by a metallopeptidase (Zmpste24 or endoprotease RAS converting enzyme 1 (RCE1)). Thus, only B-type lamins remain farnesylated, and therefore their interactions with the INM are retained. Furthermore, post-translational modifications (PTMs) of mature lamins include multiple

Figure 1



Nuclear lamins: proteins and assemblies. **(a)** The lamin protein-domains organization is conserved among species. The domains of lamin A/C, B1, B2 as well as the single *C. elegans* lamin the and frog oocyte LIII are shown. **(b)** Bacterially expressed *C.elegans* lamin assembled under low ionic strength buffers into ~8 nm thick filaments, imaged by cryo-electron tomography [46]. **(c)** Human lamin A assembled *in vitro* into paracrystalline arrays, imaged by cryo-electron tomography. These paracrystalline structures are not ordered enough to allow the structural determination of the lamin assemble. **(d)** A tomographic slice through the nuclear lamina of a nuclease treated mammalian nucleus reveals the lamin filaments meshwork. **(e)** A tomographic slice through a native nuclear lamina as observed by cryo-ET of cryo-FIB milling of a MEF nucleus, lamins filaments (arrow heads) and chromatin (arrow) are seen. **(f)** 2D averaging revealed structural classes of lamin filaments. A 3.5 nm thick rod domain (blue arrow head) decorated with globular Ig-folds (red arrow head) with a 20 nm repeat (adapted from [58•]).

phosphorylations [27] that can promote lamin solubility and delocalization of lamin A into the nucleoplasm [28,29].

Recent studies showed that lamins are involved in mechano-signaling and have a major role in force transduction [30–32]. We hypothesize that these unique properties must be reflected in the structure of lamin proteins and subsequent filament assembly. In this review, we provide a summary of lamin structures and the mechanical properties of these intermediate filament proteins.

Towards structural determination of lamins

Intermediate filaments assemble into dimers in solution. Lamin dimer analysis revealed the formation of a parallel coiled-coil structure between two monomers using metal shadowing transmission electron microscopy (TEM) [33]. Lamin dimers further assemble by head-to-tail association into long polymers, which further associate laterally into the mature filaments [34]. These assembly steps were only identified at low resolution and are still awaiting to be resolved in high resolution.

Recent biochemical analysis of *in vitro* assembled lamin A dimers and tetramers suggested that the structure of lamin dimers is affected by the chemical environment during the assembly, for example, electrolyte compositions [35••]. Linker regions within the lamin proteins can be compressed, leading to shorter repeat units. This implies that flexible linker regions allow sliding of

dimeric coiled-coil domains within tetrameric higher-order assemblies.

High-resolution structural determination is required to detect sliding of lamin dimers and tetramers. However, due to the length of lamin dimers, the coiled coil domain is ~50 nm while the tail and the head domains are not contributing to the filament scaffold, structural determination of lamin dimers is a challenging task [36]. Their tendency to rapidly polymerize makes full-length lamins as well as the intact coiled-coil domains difficult to approach in structural studies by x-ray crystallography [37]. A detailed atomic model of lamin proteins is still unavailable, however, a 'divide-and-conquer' crystallization strategy has provided insights into the organization of the lamins, by determining the crystal structure of (mostly) lamin A fragments [37,38]. The atomic structure of the Ig-fold of human lamin A/C was determined, exhibiting a globular two β-sheets structure [39,40]. The analysis of coiled-coil fragments provided insights into the intra-organization of lamin dimers [41].

The structures of lamin coiled-coil α-helical segments containing 1A, 1B and small part of 2A exemplified the interactions between lamin coil 1B to form a tetrameric assembly were recently resolved by x-ray crystallography [42••,43••]. These studies revealed the mode of interactions between the 1B coiled-coil domain of dimers to form tetramers. These interactions suggested to be conserved in other IF proteins, for example, vimentin and keratins [43••]. Surprisingly, it showed that the linkers positioned within the coiled-coil domain are folded into α-helical structures, and form a continuum of the coiled-coil structure. Furthermore, it was suggested that interdimer contacts between helix 1 promotes the antiparallel interaction of helix 2 that may promote the longitudinal assembly of lamins [42••,43••]. However, high-resolution structural analysis of lamin assemblies is required in order to confirm these interactions in higher-order lamin assemblies.

Lamin assembly was studied *in vitro* and showed to be a hierarchical process. The chemical condition to assemble the *Caenorhabditis elegans* lamin into stable filaments are established, in contrast to recombinantly purified lamin from other organisms. Hence, these filaments can be analyzed by electron microscopy approaches (Figure 1b) [44–46]. A major disadvantage of the *in vitro* structural analysis of lamins is the tendency of the mammalian lamins to assembled into paracrystalline arrays (Figure 1c), which are probably not relevant in a biological context [34]. This observation suggests that assembly of lamin filaments requires-specific conditions or maybe some factors that are found at the nuclear envelope (NE) to provide the precise environment for homogeneous lamin filament assembly (Figure 1d–f).

Architecture of lamins at the nuclear lamina

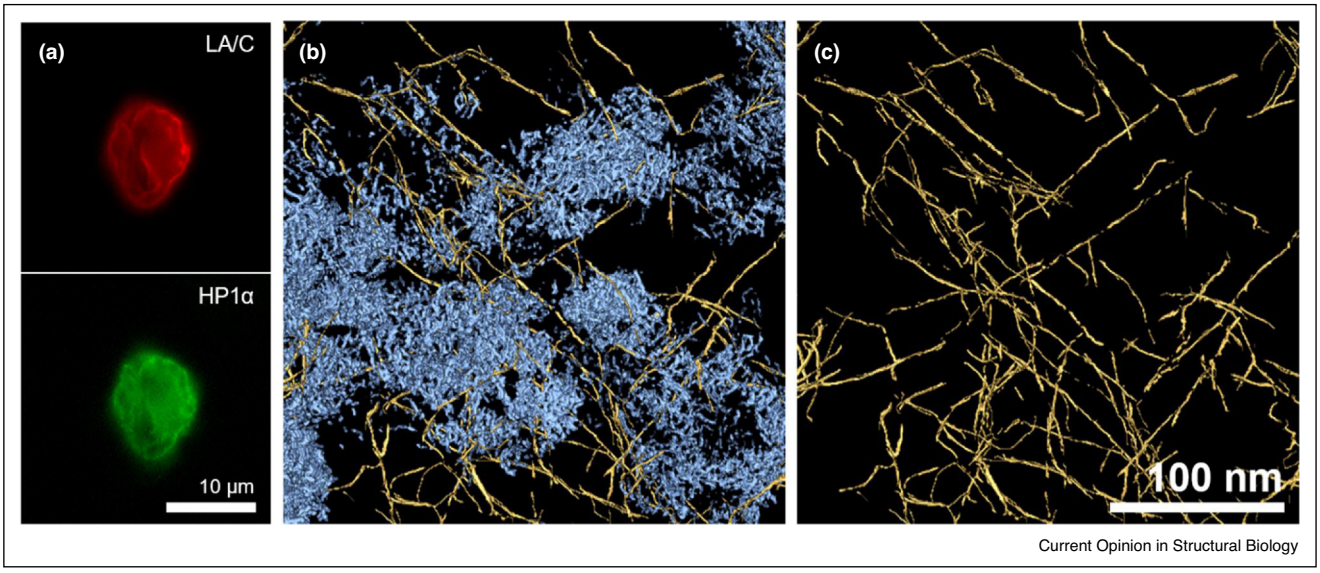
Cryo-electron tomography (cryo-ET) is a powerful technique to acquire structural insight into single non-repeating structures [47–49] and therefore became pivotal in cell biology [34,50,51]. This method enables structure determination of macromolecular complexes within a cell [52–55]. Recent technological developments allow to acquire a high-resolution snapshot of molecular processes at a specific point of time [56,57]. Therefore, cryo-ET is the method of choice to decipher the structure of lamin assemblies at close-to-native state.

Recently, we showed that lamin filaments assemble into 3.5 nm thick filaments (Figure 1f) within a ~14 nm thick meshwork layer beneath the nuclear membrane [58•]. The filaments are both highly variable in length and exhibit a short persistence length of <200 nm which hints at a large flexibility. This persistence length means that lamin filaments are more bendable than any other components of the cytoskeleton, including other IF proteins. This physical characteristics of lamins represent their unique mechanical properties that are observed when lamin filaments are subjected to external forces [59,60•].

In the nuclear lamina, the Ig-fold of lamins were seen as globular domains decorating the filaments every 20 nm. The different lamin isoforms could be partially identified using immunogold-labeling. It showed that A-type and B-type lamins form two separate meshworks, confirming previous observations obtained by structural illumination microscopy (3D-SIM) that lamin A, C, B1 and B2 form individual meshworks within the nuclear lamina [61]. An additional study using stochastic optical reconstruction microscopy (STORM) showed that the lamin meshworks are also spatially distinguished, with the lamin B1 meshwork lying closer to the membrane and the highest concentration of lamin A/C is found further towards the nucleoplasm [62•].

The nuclear lamina contains an additional set of proteins and has been shown to tightly bind chromatin [63,64]. Therefore, the nuclear lamina plays a major role in the nuclear architecture and gene expression [63,65]. The heterochromatin domains, which bind to the lamina are referred to as lamina-associated domains (LADs) and are kept in a transcriptionally repressed state to maintain genome stability [66]. It was recently shown that the interaction of lamin B1 with chromatin is synchronized with the circadian clock [67]. The interactions between heterochromatin and lamin can be observed by means of cryo-ET (Figure 2). This image reveals that the chromatin is intertwined between nuclear lamins to form a direct interaction which can resist nuclease treatments. However, studying more native and intact nuclear samples is needed to decipher the complicated interactions between chromatin and lamins.

Figure 2



Visualizing lamin-chromatin interactions. **(a)** Applying nuclease treatment to lysed cells, as described in [58•], retains heterochromatin and lamins, as revealed by visualizing HP1α and LaminA/C. **(b)** and **(c)** Segmented cryo-tomogram shows tight interactions between lamin filaments (yellow) and heterochromatin (blue).

The use of cryo-FIB (focused ion-beam) milling as a sample preparation procedure for cryo-ET allows to study any cellular structures inside the native environment of vitrified cells and multicellular samples [68,69]. By applying recent advances in cryo-FIB milling, in combination with cryo-ET [49,70,71••], it is possible to study structures in the nucleus [72–74]. FIB milling is used to create thin lamellas of cells or other structures which are too thick (>1 μm) and therefore unsuitable for native cryo-ET. Even subtomogram averaging approaches of nuclear structures like the NPC and nucleosome are feasible [73,75]. Therfore, not only lamins but all lamin-associated proteins, for example, the NPCs, the chromatin and the nuclear membranes can be studied. Thereby, combining cryo-FIB and cryo-ET allows us to study the structural interactions of the nuclear lamina with these cellular structures at high resolution. A typical view into the nuclear lamina as revealed by applying FIB-milling in conjunction with cryo-ET is shown in Figure 1e.

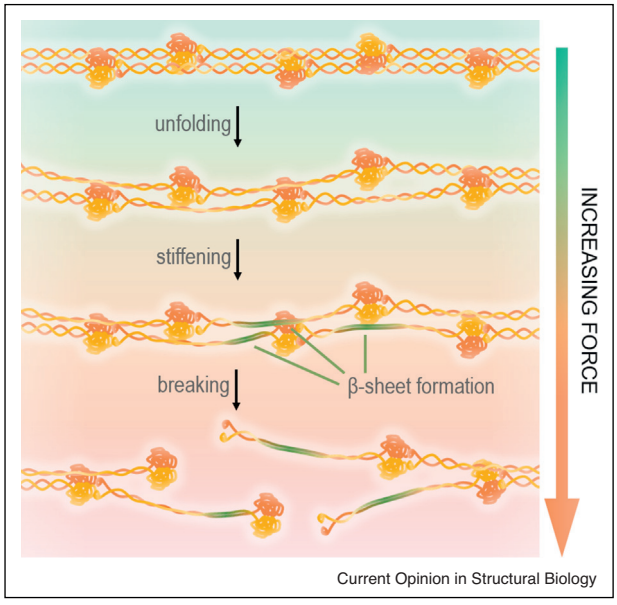
Mechanical properties of nuclear lamin filaments

Lamins contribute to the mechanical stability of the nucleus, transduce mechanical forces and biochemical signals across the NE and regulate the organization of chromatin [30,76]. Studies performed on the nucleus and entire organisms are very informative but influenced by nuclear membranes, chromatin, and surrounding cells, respectively. Such studies have assessed the changes to differing levels of lamins and NE proteins, and the underlying alterations of the physical properties [24,77]. Although chromatin plays a major role in nuclear mechanics [78], the question if lamins have special mechanical properties, is of major importance [79••].

A first glimpse into the mechanical properties of the lamins came from *in vitro* assembled macrofilaments that were subjected to external mechanical stress [59]. This macroscopic paracrystalline assemblies showed that lamins can withstand strong external forces. However, native A-type and B-type lamin filaments can only be assembled *in situ*. Such a study was recently conducted using a *Xenopus laevis* oocyte. The *X. laevis* oocyte lamin LIII revolutionized our understanding on the nuclear lamina since it was first visualized [80]. In this setup, a manually opened NE is visualized both by cryo-ET as well as by atomic force microscopy (AFM). Therefore, the mechanical properties of the lamin LIII can be directly measured within an *in situ* assembled lamina. Furthermore, the spread NE is free of chromatin, therefore only mechanical properties from the nuclear lamina are measured.

AFM-based force-extension (FE) measurements revealed that lamin filaments deform in a reversible manner under low loads (<500 pN) (Figure 3). However,

Figure 3



The lamin filaments under stress. A Model depicting the deformation of a lamin filament under external stress. Under low force the filaments are deformed by unfolding of the helical domains. This adaption to the force is reversible. When higher force is applied, the filaments stiffens by transitioning the α-helical structures to β-sheets. This is an irreversible process that may lead to the filament breakage.

when force is increased (>2 nN), lamins are stiffening substantially until the filament is failing. This suggests that lamins are a special material that is very elastic when a low force is induced but becomes a plastic stiff material when stronger forces are applied. Thus, lamins can react to force and change their behaviour dependant on the amount of force. Furthermore, molecular dynamics analysis suggested that the low force rearrangements are caused by reversible reorganization of the coiled-coil helical domains that may involve local unfolding. However, upon stronger forces lamins undergo a major irreversible transformation from an α-helix to a β-sheet that make these filaments a stiff material. This suggests that upon strong external forces lamins are 'sacrificed' by irreversible unfolding to physically protect the nucleus (Figure 3). Mechanical protection of the nucleus by the lamin meshwork are integrated at each level of the hierarchical construction of lamins, from the basic building block of the coiled-coil domains up to the higher order meshwork. Lamin filaments are extensible, strong and tough, similar to natural silk and superior to the synthetic polymer Kevlar®. Although the study was conducted on the LIII lamins, structural analysis by cryo-ET showed the similarity to mammalian lamins and the nuclear lamina meshwork of fibroblasts (Figure 3).

Concluding remarks

Lamin filaments are essential components of the cell nucleus. These thin IF proteins, ~3.5 nm in diameter, are assembled underneath the nuclear envelope although a pool of A-type lamins is located in the nucleoplasm and was found to interact with specific binding partners, such as LAP2α [17]. These lamin A/C structures are more dynamic than the ones at the peripheral lamina and likely have unique structural assembly states and functions, fundamentally different from those of the peripheral lamina However, the *in vitro* assembly conditions for mammalian lamins have not been found yet. Surprisingly, lamin filaments have unique physical properties that may have evolved to fulfill their major task — to provide unique mechanical properties to the nucleus. High-resolution information is crucial to unveil the alteration of the nuclear lamins organization at the lamins, nuclear interior and in health and disease, but also when nuclei experience external forces, for example, when cell pass through narrow passages [24,25]. Changes in the lamina during the cell cycle may also require the correlation between structural analysis and cell stages. With recent advancements in cryo-ET as well as complementary modalities, these fundamental aims are within reach.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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